CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH
SUMMARY OF TOXICOLOGY DATA

Diflubenzuron (also called Dimilin, Du 112307)

Chemical Code # 001992, Tolerance # 00377
SB 950 # 244

October 12, 1989
Revised January 22, 1991, April 9, 1992 and 23 June 1997

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, possible adverse effect
Oncogenicity, rat:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, no adverse effect
Reproduction, rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time.

Toxicology one-liners are attached.

All relevant record numbers through 134787 were examined as of 6/23/97. In addition, record numbers > 900,000 were considered.

10/12/89 Summary prepared by C. Aldous; revised 1/22/91 and 4/9/92 by J. Gee; revised by P. Iyer 6/23/97

In one-liner identifying numbers below:
** indicates an acceptable study.
Bold face indicates a possible adverse effect.

These pages contain summaries only. Individual worksheets should be reviewed as they may contain additional effects.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

COMBINED, RAT

****377-055 010464** "Oncogenicity study in rats: Diflubenzuron". Hazleton (Vienna, VA): Project No. 553-122. April 6, 1984. 2-year treatment of CD rats, 100/sex for controls, and 50/sex in treatment groups of 156, 625, 2500, and 10,000 ppm diflubenzuron (Tech., 97.6% purity, dosages adjusted to provide 100% of target ppm levels of A.I.). Oncogenicity NOEL $\geq$ 10,000 ppm, i.e. **no treatment effect on neoplasia. Possible adverse effect**: mild hemolytic anemia. No NOEL was determined, however the LDT (156 ppm) is a defensible NOAEL. At 156 ppm, males and females had increased sulfhemoglobin (SulfHb) and methemoglobin (MetHb) at one or more sampling periods. The degree of pigmentation of splenic macrophages was seemingly slightly increased at this dosage and above in both sexes. Common findings at 625 ppm included increased pigmentation of macrophages in spleen and liver, and erythroid hyperplasia in bone marrow. Reductions in common hematological measures (HCT, Hb, RBC count), as well as increases in reticulocyte counts, were generally limited to the upper two dosages. For reasons listed in the 10/12/89 review, this study is considered to fill the **Rat Combined Study** data requirement. A. Apostolou, 7/15/85; C. Aldous, 10/12/89.

056-058 010465, 010466, 010467 Individual data for 055:010464, above.

377-023 975383 "Effects of Du 112307 in dietary administration to rats for 104 weeks". Huntingdon Research Centre, Report PDR 171/75945, date presumed from reference in a commentary (074:037643) to be Jan., 1976. 60 CD rats/sex/group dosed with 0, 10, 20, 40, or 160 ppm Du 112307 [diflubenzuron] in diet for up to 104 wk (45/60 of each sex/group designated for oncogenicity study). **Possible adverse effect indicated** (increased methemoglobin at 160 ppm). **No oncogenicity indicated** under treatment conditions. Study **unacceptable, not upgradeable**: No MTD achieved (or even approached); inadequate numbers of animals, coupled with high mortality, limited statistical power; inadequate histology; inadequate clinical chemistry and hematology protocol. CDFA review by A. Apostolou, 7/10/85 (one-liner by C. Aldous, 9/11/89).

024 025225 Continuation of 023:975383, above (individual data).

074 037643 Commentary on 023:975383, above. No data. No impact on CDFA review status. C. Aldous, 9/11/89.

077 037669 Commentary on 023:975383, above. No impact on CDFA review status. No CDFA worksheet. C. Aldous, 9/19/89.

CHRONIC TOXICITY, RAT

(See Combined, Rat, above)

CHRONIC TOXICITY, DOG

****377-078 037670** "Diflubenzuron: 52 week oral toxicity study in dogs". Inveresk Research International, Feb. 1985. (IRI Project No. 630146). Beagle dogs, 12/sex in controls, and 6/sex/group in treatment groups of 2, 10, 50, and 250 mg/kg/day. Dosage by gelatin capsule with diflubenzuron, technical grade - air milled powder, 97.6% purity. **Possible adverse effect:** hemolytic anemia. NOEL = 2 mg/kg/day (females). No NOEL determined in males. NOELs based on pigmentation of liver macrophages and Kupffer cells, which appeared in mild degree in males down to the lowest dose tested. Other sensitive indicators of hemolytic anemia were the altered

hemoglobins: MetHb and SulfHb were elevated at 10 mg/kg/day and above in both sexes. Other major findings were less sensitive responses to hemolytic anemia, including changes generally limited to the highest dosages in standard hematology parameters (RBC count, Hb, HCT), elevated reticulocyte counts, presence of Heinz bodies, slightly elevated plasma bilirubin, and enlarged spleen. Study is **acceptable**. C. Aldous, 9/8/89.

ONCOGENICITY, RAT

(See Combined, Rat, above)

ONCOGENICITY, MOUSE

Note that the 1984 Huntingdon study involved much higher dosages than the 1978 Huntingdon study. The more recent study indicated **no** oncogenicity, therefore the overall conclusion is that there is **no** oncogenic effect in the mouse. C. Aldous, 10/3/89.

****377-060 012114** "The effect of diflubenzuron given by oral administration with the feed on toxicity and tumor development in male and female HC/CFLP mice". Huntingdon Research Centre, 5/15/84. CFLP mice were fed 0, 16, 80, 400, 2000, or 10,000 ppm tech. diflubenzuron (97.6%) in diet for up to 91 wk. There were 104 controls/sex assigned for the full duration of study, compared to 52/sex/group in treated mice. In addition, mice assigned for interim sacrifices numbered 24/sex in controls and 12/sex in treated groups at each of weeks 26, 52, and 78. **No oncogenicity response.** No NOEL was observed: at 16 ppm there were statistically significant elevations of MetHb in males, and significant elevations of SulfHb in both sexes. Major findings of the study were consistent with mild hemolytic anemia. In addition to altered hemoglobins mentioned above, signs seen at relatively low dosages included the following, with LELs of 80 ppm in males: cyanosis (blue/gray coloration, seen during clinical observations), presence of Heinz bodies, and enlarged spleens. The latter three findings were seen in females at 400 ppm upward. Lower HCT and lower RBC counts were observed in 10,000 ppm females, and lower RBC counts were also seen in 2000 and 10,000 ppm males: no other consistent changes in HCT, Hb, or RBC counts were seen below 10,000 ppm in either sex. Other signs related to hemolysis, generally limited to higher dosages, included extramedullary hematopoiesis in spleens and livers, siderocytes in spleen, and pigmented Kupffer cells in liver. Hematologic findings are **possible adverse effects**: it is recommended that risk assessment consider that dose-response curves were <u>not</u> steep, and that even the highest dosages were reasonably well tolerated. **Acceptable as an oncogenicity study**. A. Apostolou (brief review on 7/12/85), current review by C. Aldous, 10/3/89.

061-066 012115-012120 Individual data for 060:012114, above.

377-077 037665 "Tumorigenicity of Du 112307 to mice: Dietary administration for 80 weeks". Huntingdon Research Centre, 23 Dec. 1975. Doses of 0, 4, 8, 16, or 50 ppm in diets of 52/sex/group CFLP Swiss-derived mice for 80 wk. No adverse effect indicated: (An increase in lymphoreticular tumors was originally observed in females. On examination of fresh sections of relevant tissues (in 077:037667, below), no statistically significant differences were obtained for lymphoreticular tumors). **Unacceptable, not upgradeable**: Dosage range was apparently far below an MTD. No CDFA worksheet; C. Aldous, 9/06/89.

377-025 025228 "Tumorigenicity of Du 112307 to mice, dietary administration for 80 weeks. Revaluated [sic] pathological data." 5 April, 1977 addendum to study 077:037665, above. Notations of possible tumors "of unknown type" in two mice. No significant value in interpretation of 077:037665. No CDFA worksheet. C. Aldous, 10/3/89.

377-077 037667 (re-reading of slides, re-evaluation of statistical data). "Tumorigenicity of Du 112307 to mice, dietary administration for 80 weeks. Addendum: Histological examination of additional tissues". Huntingdon

Research Centre, Aug. 12, 1977. Considered as part of 077:037665, above.

377-077 037666 Re-evaluation of slides for study 077:037665, above. No major changes: no change at all in incidence of female lymphoreticular tumors. C. Aldous, 8/28/89.

377-077 037668 Comment by Dr. O. R. Offringa of Philips-Duphar: mouse study (077:037665) dose levels were established on the basis of a limited 6-week study in male mice, in which only livers were examined microscopically. Foci of liver cell necrosis were found in 3/8 mice at 50 ppm, but not in other groups. CDFA conclusion: inadequate basis for dose justification. C. Aldous, 8/28/89 (no review worksheet).

026 975384 Exact duplicate of 077:037668.

023 066122 A discussion of statistical techniques using data from 077:037665 as an example (no CDFA review). C. Aldous, 9/19/89.

023 975387 "Review of blood smears from CF-LP mice in an 80-week bioassay of dimilin". This second examination of blood smears of study 077:037665 reportedly did not change the evaluation with respect to leukemogenic effect of test article: investigators did not find a treatment effect on leukemias as a result of this re-examination. Data apparently was examined by A. Apostolou, but no written review was generated, nor does one appear to be needed (C. Aldous, 9/19/89).

023 975391 "Review of microscopic slides from liver and lung of control or dimilin-treated (16 ppm, 50 ppm) female mice". These tissues were examined from animals in study 077:037665. No treatment-related tumors were found. This record does not impact disposition of study. No worksheet. Aldous, 9/20/89.

023 975392 Another re-examination of lung and liver slides from study 077:037665. Same outcome. No worksheet. Aldous, 9/20/89.

023 975393 "Pathology review and analysis of 80-week bioassay of dimilin in male CF-LP mice". Examination of brain, testes, and epididymides of male mice from study 077:037665, which were not routinely examined for the initial report. No treatment effects indicated. No worksheet. Aldous, 9/20/89.

023 975390 "Critique of EPA's Carcinogen Assessment Group's Preliminary Report on Dimilin". Statement by Clement Associates, Inc. in response to claims by CAG that dimilin may have elicited lymphoreticular tumors in study 077:037665. Arguments against the CAG conclusion included: (1) lymphosarcomas should not be considered separately from other lymphoreticular tumors, (2) the CAG conclusion of a decreased latency of such tumors was invalid, (3) statistical analyses should have involved the Bonferroni inequality, (4) there was no positive dose-relationship for lymphoreticular tumors for the two highest dosages in the female mice, (5) historical control data were not fully considered. The statement also rebutted the CAG conclusion that dosages were below the MTD, and other issues were discussed. Since CDFA does not identify an oncogenicity effect, and since CDFA has considered arguments regarding adequacy of dosage already, no CDFA worksheet is appropriate. This statement has no impact on disposition of the cited mouse study. C. Aldous, 9/19/89.

074 037642 Comment by T. J. Ennis on mouse oncogenicity study 077:037665. No new data were presented. No impact on CDFA evaluation. C. Aldous 9/11/89.

REPRODUCTION, RAT

**377-132 134787 "Diflubenzuron Technical: The effect on reproductive function of two generations in the rat" A.J. Brooker, Huntingdon Research Centre, 2/7/95. Diflubenzuron, 97.1% purity (batch no: FUX 021000/No. FUN 91A10A) was administered in the diet at fixed concentrations of 0 (control), 500, 5000 and 50000 ppm through two generations (1 litter per generation) of Crl:CD (SD)BR VAF/Plus strain rats. F0 animals (32/sex/group) and F1 animals (28/sex/group) were treated via diet, during the premating period (F0 -10 weeks; F1 - 12 weeks) through pregnancy, and continued through till termination after the respective litters were weaned. Reduced body weight gain was noted for the F0 animals in the 50000 ppm group through the premating period. No effects on reproductive parameters were observed, however the mean pup weight of the treated F1 offspring was lower than controls. Anemia characterized by lower PCV, Hb, RBC counts and polychromasia in both generations was observed. Anisocytosis in the treated F0 animals and Howell-Jolly bodies in the treated F1 animals (particularly females) were also noted. MCV and MCHC was increased at 5000 and 50000 ppm and increased methemoglobin concentration was noted in all treated groups. A lso, pigmented Kupffer cells and splenic hemosiderosis were noted in all treatment groups of F0 animals and at 5000 and 50000 ppm in F1 animals. Centrilobular hepatocyte enlargement was noted for for both 5000 and 50000 ppm groups with minimal centrilobular hepatocyte vacuolation in liver of F1 male rats. Accordingly a NOEL for parental toxicity is not available from this study. However, although no reproductive parameters were affected, due to decreased pup weights the NOEL for reproductive effects = 5000 ppm. Acceptable (P. Iyer, 6/23/97).

377-025 975397 "Effect of Du 112307 on reproductive function of multiple generations in the rat". Huntingdon Research Centre, report date not given: CDFA receipt date, 1980. CD rats were maintained on diets containing 0, 10, 20, 40, or 160 ppm of (apparently) tech. diflubenzuron for 3 generations. No adverse effects indicated. A brief review by A. Apostolou on 7/11/85 had indicated a "possible adverse effect" on pup survival. Data were re-examined by C. Aldous on 9/20/89, resulting in the determination that the quality of data was too poor to warrant a "possible adverse effects" designation. Re-evaluation confirmed Dr. Apostolou's conclusions about major weaknesses of the study: dosage levels were not justified, disease and/or other management problems resulted in poor general survival, and test article was not well characterized. Study **unacceptable, and not upgradeable**. C. Aldous, 9/20/89.

377-079 037671 "Effect of dietary administration of DU 112307 on reproductive function of one generation in the rat". Huntingdon Research Centre, 11/20/78. DU 112307 [= diflubenzuron], purity not given, in diets of CFY rats at 0, 1000, or 100,000 ppm for 9 weeks prior to mating, with treatment continuing through weaning of F1 pups [end of study]. This was a single-generation study, with 20/sex/group mated 1 to 1 for one littering period. F1 offspring were sacrificed at weaning. Study included selected microscopic examination of weanlings, as well as microscopic examinations of adults. No NOEL for parental toxicity. Most prominent signs in adults were associated with hemolytic anemia, including reductions of hematology measures such as HCT, Hb, RBC count; also evidence of damage to hemoglobin: e.g. increased MetHb and apparent SulfHb. Also, pigmented Kupffer cells and splenic siderosis were dose related in adults of all treatment groups of both sexes. No adverse effects on reproduction or on litter survival or development. NOEL for histological findings in weanlings was 1000 ppm (slight hepatocellular hypertrophy). **Not acceptable, not upgradeable; no adverse effects indicated.** C. Aldous, 9/11/89.

025 975398 Interim report of study 079:037671, above.

377-028 025244 "Selected toxicological studies of dimilin in weanling male rats". EPA Exposure Assessment Division, Environmental Monitoring Systems Laboratory, Las Vegas, NV, Feb., 1980. This is a brief report assessing the following effects on weanling Long-Evans rats treated for 14 to 96 days at 0, 15, 150, or 300 mg/kg/day: plasma testosterone levels; growth of testes, prostate, seminal vesicles, and adrenal glands; and histological examinations of these tissues. The report provides summary tables for body weight, organ weight, and plasma testosterone levels. Organ weights and microscopic appearances of tissues did not change, however a **transitory but substantial decrease in plasma testosterone** was observed in young rats. Differences between

male gonadal effects of diflubenzuron and another substituted phenylurea compound were discussed (see review). C. Aldous, 9/21/89.

377-028 975427 "An evaluation of the effect of diflubenzuron on animal serum testosterone levels". This is a review of literature on effects of diflubenzuron on serum testosterone levels in various species. Except for the one study above (028:025244), there is no indication of treatment effects of diflubenzuron on serum testosterone levels in any of the 5 species for which studies were reported in the literature search. Another strain of rat (Sprague-Dawley) did not indicate such changes at comparable dosages. The reviewer (G. M. Booth) determined that the overall data base did not indicate a concern about testosterone effects. No CDFA worksheet. C. Aldous, 9/21/89.

377-124 113678 Protocol for a rat reproduction study to be conducted at Huntingdon Research Centre in England. For comments, see file P920408. Gee, 4/9/92.

TERATOLOGY, RAT

** 377-112 087996 "Diflubenzuron Oral (Gavage) Rat Teratology Limit Study." (Kavanagh, P., Toxicol Laboratories Limited, Eng., Duphar Report # 56645/68/1987, 4/88) Diflubenzuron, batch FL66/FUN80D21D, 97.6%, was given by oral gavage to Crl : CD (SD) BR female rats at 0 (1% w/v gum tragacanth) or 1000 mg/kg/day (limit dose) on days 6 - 15 of presumed gestation, 24 per group. There were no clinical effects, effect on body weight, food consumption or fetuses due to treatment. The incidence of minor skeletal abnormalities was slightly increased (20.2% in control group versus 26.3% in treated group) but it was within historical control range. No adverse effect. Maternal = Developmental NOEL $\geq$ 1000 mg/kg/day. **Acceptable**. Gee, 12/28/90.

377-075 037644 "Effect of Du 112307 on pregnancy of the rat". Huntingdon Research Centre, 4 Feb., 1975. CD rats treated with 0, 1, 2, or 4 mg/kg/day on days 6-15; 20 dams/group. No adverse effects indicated: no maternal toxicity, nor developmental effects observed. Study **unacceptable, not upgradeable**: dosages were apparently orders of magnitude below maternally toxic range. C. Aldous, 8/28/89.

042 002003 A 1-paragraph reference to 075:037644. This entry was given a brief worksheet by A. Apostolou, 7/10/85. (review says negative for teratology).

TERATOLOGY, RABBIT

** 377-112 087997 "Diflubenzuron Oral (Gavage) Rabbit Teratology Limit Study." (Kavanagh, P., Toxicol Laboratories Limited, U. K., Duphar Report No. 56645/79/87, 4/88) Diflubenzuron, batch FL66/FUN80D21D, 97.6%, was given by oral gavage at 0 (vehicle) or 1000 mg/kg/day (nominal, limit test) to groups of 16 does, days 7 - 19 of gestation. No clinical signs, effect on body weight or food consumption were reported. The incidences of major and minor external, visceral and skeletal findings were not affected by treatment. No adverse effect. **Acceptable.** Gee, 12/28/90.

377-025 975395 "Effect of Du 112307 on pregnancy of the New Zealand White Rabbit". 7 Feb., 1975, Huntingdon Research Centre. A study with dosages of 0, 1, 2, and 4 mg/kg/day by gavage on days 6-18 to 13 dams/group. No effects on dams or fetuses. **Unacceptable, not upgradeable:** dosages not justified, and apparently far below justifiable range. CDFA worksheet by Apostolou, 7/11/85.

377-074 037642 Mention by T. J. Ennis of a hamster teratology study (see p. 2, last section of volume). No data were presented. No hamster teratology study for diflubenzuron has yet been submitted to CDFA . C. Aldous 9/20/89.

GENE MUTATION

** 377-114 095255 "Study to Examine the Possible Mutagenic Activity of Diflubenzuron in the Ames Salmonella/Microsome Assay." (Koorn, J. C., Duphar B. V., The Netherlands, Report No. 56645/74/90, 5/11/90) Diflubenzuron, 96.9%, was tested with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 by plate incorporation. Concentrations used were 0 (DMSO), 8, 40, 200 and 1000 µg/plate. Triplicate plates with two trials were used with and without rat liver activation. No evidence of an increase in reversion rate. **Acceptable.** (Gee, 1/18/91)

377-073 037678 "Further Mutagenicity Studies on Pesticides in Bacterial Reversion Assay Systems." (Publication in: Mutation Research 116: 185 - 216 (1983), Moriya et al.) Diflubenzuron was one of 228 pesticides tested with Salmonella typhimurium strains; TA1535, TA1537, TA1538, TA98 and TA100; tested to 5000 µg/plate; data for diflubenzuron as "-" only - no data; **unacceptable** (no data, no purity, no concentration given). Gee, 9/29/89.

377-074 037632 "Results of Testing USDA Compound WRRC-153-10-TOX, with and without Metabolic Activation, in the Mouse Lymphoma TK+/- ; TK-/- Mutagenesis System." (SRI, 4/25/77) Compound WRRC-153-10-TOX is never defined in the report. Since the study was negative and not acceptable as submitted (primarily because the test material was not described), no worksheet was prepared. Otherwise, the study appears to have been well conducted. Gee, 9/29/89.

026 066123 Exact duplicate of 074:037632. Retain both copies.

377-073 037648 "Mutagenicity Evaluation of Diflubenzuron Technical Batch FL 44/605201: Final Report." (Litton Bionetics, 5/17/77) Diflubenzuron technical, no purity given, batch FL 44/605201; tested with Salmonella typhimurium strains, TA1535, TA1537, TA1538, TA98 and TA100 and with Saccharomyces cerevisiae strain D4 for gene mutation; 0 (DMSO), 0.1, 1.0, 10, 100 or 500 µg/plate in the plate incorporation assay; single plate per concentration, single trial; marginal evidence of cytotoxicity with TA1537 without activation at 100 and 500 µg/plate; Aroclor-induced male rat liver S9 for activation; no evidence of an increase in reversion rate; **not acceptable and not upgradeable** (single plate per concentration). Gee, 9/22/89.

377-026 975407 Exact duplicate of 073:037648.

377-073 037651 "Activity of TH-6040 in the Ames Salmonella typhimurium Mutagenesis Assay." (Laboratory not stated, 8/6/76) TH-6040, no purity or grade, tested with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 in the spot test at 1000 µg/spot; single plate, single trial; no evidence of an increase in reversion rate with or without rat liver activation; AAF as only positive control was effective in a concentration-dependent manner; **unacceptable and not upgradeable**. Gee, 9/28/89.

025 040997 Exact duplicate of 073:037651. Retain both copies.

377-073 037652 "Mutagenicity Tests of Diflubenzuron in the Micronucleus Test in Mice, the Mouse Lymphoma Forward Mutation Assay, and the Ames Salmonella Reverse Mutation Test." (Publication in: Mutation Research 66: 45-53 (1979) by Macgregor et al.) Diflubenzuron, technical grade (>99%) and a 25% wettable powder (commercial formulation, W-25); tested with Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 with and without rat liver activation at 100 µl mix per ml; duplicate plates; 0, 10, 100 or 1000 µg/plate technical; 19, 186, 1860 of wettable powder as µg diflubenzuron per plate; positive controls active; no evidence of increase in reversion rate reported; **unacceptable** (inadequate number of replicate plates, concentrations used not justified, no individual data), not upgradeable. Gee, 9/28/89.

026 975430 USDA summary of studies, reference to 073:037652, above.

377-073 037652 "Mutagenicity Tests of Diflubenzuron in the Micronucleus Test in Mice, the Mouse Lymphoma Forward Mutation Assay, and the Ames Salmonella Reverse Mutation Test." (Publication in: Mutation Research 66: 45-53 (1979) by Macgregor et al.) Diflubenzuron, technical grade (>99%) and a 25% wettable powder (commercial formulation, W-25); tested with mouse lymphoma L5178Y; selection with trifluorothymidine at 1 µg/ml; with and without activation with homogenate from male Swiss-Webster mice (induction not mentioned); tested at 0 (DMSO), 1.17, 4.69, 18.75, 37.5, 75, 150 or 300 µg/ml (presumably technical grade but not stated), duplicate cultures; EMS (500 µg/ml) and DMN (3×10^{-4} M); incubated for 4 hours without activation and 2 hours with activation; 3 day expression time; precipitation without activation at 150 and 300 µg/ml; no evidence of an increase in forward mutations; **unacceptable** (number of plates not given for colony formation, use of uninduced mouse liver activation not discussed; methods primarily by citation; data not identified as technical or wettable powder used), possibly upgradeable with a full report. Gee, 9/28/89.

023 975408 Draft of 073:037652, with handwritten corrections. Multiple copies in this volume. Retain at least one volume of this record.

026 033584 Brief synopsis of 073:037652, above.

CHROMOSOME EFFECTS

** 377-089 049902 "Mutagenicity Evaluation of Diflubenzuron Technical in an in vitro Cytogenetic Assay Measuring Chromosome Aberration Frequencies in Chinese Hamster Ovary (CHO) Cells: Final Report." (Hazleton Biotechnologies Corporation, The Netherlands, 6/86). Diflubenzuron, technical grade, no purity stated; tested with Chinese hamster ovary cells (CHO-WBI) in culture at 0 (DMSO and untreated), 100, 150, 200 or 250 µg/ml with and without Aroclor-induced rat liver activation (purchased from Litton Bionetics); incubated for 9 or 16.5 hours without activation; incubated 2 hours with activation, based on a preliminary trial in which cell cycle time was measured. Duplicate cultures with 100 cells from each scored for chromosomal aberrations. No evidence of induction of aberrations. **Acceptable** study. Gee, 9/22/89.

377-073 037647 "Mutagenic Study with TH 6040 in Albino Mice." (IBT, No. 622-05068, 7/23/74) [Status of study unknown] TH 6040, diflubenzuron, purity not stated; given by intraperitoneal injection at 0 (corn oil), 1000 or 2000 mg/kg to 12 male mice (Charles River albino) per group (doses selected based on a preliminary study to 4000 mg/kg at which dose 0/4 died but animals were "hypoactive"); mated 1:3 per week for 6 consecutive weeks; resorptions were counted as early or late; report indicates no evidence of a dominant lethal affect but is **unacceptable**, and not upgradeable without indication of the report being validated in EPA audit of IBT studies. Gee, 10/2/89.

025 975405 Exact duplicate of 073:037647. Retain both copies.

027 025242 Two - sentence reference to 073:037647, above.

377-073 037652 "Mutagenicity Tests of Diflubenzuron in the Micronucleus Test in Mice, the Mouse Lymphoma Forward Mutation Assay, and the Ames Salmonella Reverse Mutation Test." (Publication in: Mutation Research 66: 45-53 (1979) by Macgregor et al.) Diflubenzuron, technical grade (>99%) and a 25% wettable powder (commercial formulation, W-25); male Swiss-Webster mice were given two doses at 30 or 6 hours before necropsy; doses were 0 (corn oil), 15, 150 or 1500 mg/kg p.o.; TEM as positive control; 5 males per group; micronucleated polychromatic and normochromatic erythrocytes scored with 1000 polychromatic erythrocytes; no significant increase in micronucleated cells; table 4 gives individual data but does not indicate which are for 30 and which for 6 hours; **unacceptable, not upgradeable** (use of single sex, use of Schmid's protocol with no sacrifice 24 hours

after second dosing, inadequate number of animals, no justification of dose selection). Gee, 9/28/89.

DNA DAMAGE

** 377-114 095256 "Evaluation of DNA Repair Inducing Ability of Diflubenzuron in a Primary Culture of Rat Hepatocytes (With Independent Repeat)." (Enninga, I. C., RCC NOTOX B. V., The Netherlands, 56645/114/90, 7/3/90) Diflubenzuron, 96.9%, was tested with primary rat hepatocytes for induction of unscheduled DNA synthesis. Concentrations used were 0 (DMSO), 1, 3, 10, 33, 100, 333 and 1000 µg/ml, 18 hour incubation. Triplicate cultures were used in each of two trials. Fifty cells per coverslip per concentration were scored to determine the net nuclear count by autoradiography. Cytotoxicity was also determined by trypan blue dye exclusion. Individual data were included. No evidence for the induction of unscheduled DNA synthesis. **Acceptable.** (Gee, 1/18/91)

377-073 037649 "Evaluation of Diflubenzuron in vitro Malignant Transformation in BALB/3T3 Cells: Final Report." (Litton Bionetics, Maryland, 8/2/77, Project No. 2688) Diflubenzuron, no purity or grade stated; tested with BALB/3T3 cells for transforming ability at 0 (DMSO), 0.02, 0.039, 0.078, 0.156 or 0.312 mg/ml, 10 plates per concentration, 48 hour exposure followed by 3-4 weeks additional incubation; no activation included; no evidence of type II or III foci; **unacceptable and not upgradeable** (a number of plates lost to contamination, no activation included). Gee, 9/22/89.

026 975406 Exact duplicate of 073:037649. Retain both copies.

377-073 037650 "Evaluation of Diflubenzuron - Unscheduled DNA Synthesis in WI-38 Cells: Final Report." (Litton Bionetics, 8/2/77) Diflubenzuron, no grade or purity stated; tested with WI-38 at 0 (DMSO), 50, 100, 500 or 1000 µg/ml, 1.5 hours incubation; uninduced mouse liver for activation (no justification); cells in G_1 by confluency and addition of hydroxyurea; tritiated thymidine-labelled DNA extracted and quantitated and counted; no evidence of unscheduled DNA synthesis; **unacceptable and not upgradeable** (single sample per concentration, no justification for concentrations selected, no passage number, positive controls marginally effective by criteria in report). Gee, 9/22/89.

377-073 037659 "Absence of Transformation by Diflubenzuron in a Host-Mediated Transplacental Carcinogen Assay." (Texas A&M, Publication in: Bull. Environm. Contam. Toxicol. 25: 252-256 (1980), Quarles et al.) Diflubenzuron, no purity stated; injected intraperitoneally into pregnant hamsters on the tenth day of pregnancy at 0 (DMSO or untreated), 1.0, 20 or 50 mg/100 g body weight; 3 days later (13th day of pregnancy), animals were sacrificed and fetal cultures established; transformation assayed by growth in 0.3% agar; 10 - 20 dishes examined; negative for transformation under the assay conditions; positive controls (benzo(a)pyrene and dimethylnitrosamine) were effective; **unacceptable and not upgradeable** (summary report, inadequate number of animals, doses given not justified, no evidence of fetal exposure) Gee, 9/29/89.

GENE MUTATION STUDIES ON METABOLITES OF DIFLUBENZURON

377-074 037626 "Screening of Selected Thompson Hayward Chemicals for Activity in the Ames Salmonella Mutagenicity Test." (University of Kentucky, H. W. Dorough, 10/7/77) Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, TA100 and TA1978. Three compounds were tested: 4-chlorophenylurea, 4-chloroaniline and 2,6-difluorobenzoic acid. Spot test at 1000 µg/spot and plate incorporation at 0 (DMSO), 10, 100, 500 or 1000 µg/plate, two replicates at different times, with TA100 and TA98 with and without rat liver activation; a further assay was run with TA98 up to 4000 µg/plate. Cytotoxicity by zone of inhibition in the spot test. Positive dose response with 4-chloroaniline with TA98 only in the presence of activation and confirmed in a second trial. Considered

supplementary data. Gee, 9/29/89.

025 975399 Exact duplicate of 074:037626. Retain both copies.

025 975404 Appears to be a duplicate record number for 025:975399.

377-073 037656 "Metabolism of Diflubenzuron by Soil Microorganisms and Mutagenicity of the Metabolites." (Department of Microbiology, Louisiana State University, publication in: Pesticide Biochemistry and Physiology 10: 174-180 (1979) Diflubenzuron (purified by crystallization from ethanol) and metabolites of soil organisms tested for mutagenicity with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 without activation; metabolites were 4-chloroaniline, 4-chlorophenylurea, 4-chlorophenol, 4-chloroacetanilide and 2,6-difluorobenzoic acid; single plate per concentration; the numbers of colonies are reported as net with the spontaneous revertants subtracted and not given in the publication. No adverse indicated: the only suggestion of a positive result was with 2,6-difluorobenzoic acid, for which there originally appeared to be an increase in revertant colonies. Upon further testing, it was determined that most of these colonies were non-revertants. Gee, 10/2/89.

377-073 037658 "Metabolism of dimilin by microorganisms". (Thesis by S. L. Seuferer, Jr. to Louisiana State Univ., Dec. 1977). An apparently positive Ames test was reported here. The latter part of this record presents commentary by reviewing scientists, who were very critical of the quality of the Ames test performance. The publication of the data by Seuferer following additional testing is 073:037656, above.

377-073 037646 Copy of the last page of 073:037658, above.

377-074 037630, 037631 Overview of biological effects of dimilin, including brief summaries of three studies reported elsewhere in this tox summary. No CDFA review, C. Aldous, 10/10/89.

377-025 975401 "Mutagenicity Evaluation of 2,6-Difluorobenzoic Acid: Final Report." (Litton Bionetics, 10/77, project 20838) 2,6-Difluorobenzoic acid, no purity stated, white crystals; tested with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 and with Saccharomyces cerevisiae strain D4; with and without male Sprague-Dawley rat liver activation, equivalent to 25 mg wet weight liver per plate, plate incorporation assay; concentrations of 0 (DMSO), 0.1, 1.0, 10, 100 or 500 µg per plate, single plate per concentration, single trial; no clear cytotoxicity at high concentrations; **supplementary data** on metabolite. Deficiencies: single plate, concentrations not justified. Gee, 10/11/89.

377-025 975402 "Mutagenicity Evaluation of 4-Chlorophenyl Urea: Final Report." (Litton Bionetics, 10/77, Project 20838) 4-Chlorophenyl urea, white powder, metabolite of diflubenzuron; tested with Salmonella typhimurium, strains TA1535, TA1537, TA1538, TA98 and TA100 and Saccharomyces cerevisiae, strain D4; with and without activation; male Sprague-Dawley rat liver induced with Aroclor 1254, equivalent to 25 mg wet weight/ml mix added 0.5 ml per plate; tested at 0 (DMSO), 0.1, 1.0, 10, 100 or 500 µg/plate in the plate incorporation assay, 1 plate per concentration; single trial; no increase in reverse mutation reported; no conclusive evidence of cytotoxicity; **supplementary data** on metabolite. Deficiencies: single plate per concentration and concentrations not justified. Gee, 10/11/89.

377-025 975403 "Mutagenicity Evaluation of 4-Chloroanilin: Final Report." (Litton Bionetics, 9/77, project 20838) 4-Chloroanilin, white powder, no purity stated; metabolite of diflubenzuron; tested with Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 and Saccharomyces cerevisiae D4; with and without male Sprague-Dawley rat liver activation, Aroclor induced, homogenate equivalent to 25 mg liver wet weight; plate incorporation assay; tested at 0 (DMSO), 0.1, 1.0, 10, 100 or 500 µg/plate, single plate per concentration, single trial; positive controls active; no evidence of cytotoxicity; no increase in reversion rate reported; **supplementary data** on metabolite. Deficiencies: single plate, concentrations not justified. Gee, 10/11/89.

NEUROTOXICITY

Studies not required at this time

SPECIAL STUDIES

377-026 975396 "Biochemical effects of diflubenzuron on mouse embryos". Special study by Dept. of Zoology, Brigham Young University, 6/11/77. Labeled, purified diflubenzuron (50 ppm) was fed in chow to pregnant females or lactating females. Result: no detectable uptake of label into fetuses, and no uptake into lactating pups. Also, no changes in components of connective tissues, which changes might have been suspected with this compound, which is an effective chitin synthetase inhibitor in arthropods. Investigators summarized: "Specifically, sulfation of chondroitin was not inhibited and incorporation of glucosamine into glycosaminoglycan molecules was normal during the treatment". No worksheet. One-liner by Aldous, 9/20/89.

377-015 975423 "Test for estrogenic activity in female mice". Special study by Institute of Biological Sciences. Intact female mice, aged 19-21 days, were injected with TH-6040 (synonym for diflubenzuron: grade of test article not specified) subcutaneously daily for 3 days. Animals were killed, and relative weights of uteri were determined to evaluate possible uterotrophic effects. No changes in uterine weight were noted at 5 ug/mouse total dose. For comparison, 0.05 mg estrone markedly increased uterine/body weight ratios. Thus the study was negative, and demonstrated that diflubenzuron was less than 1/100 as potent as estrone in this assay. No worksheet. One-liner by Aldous, 9/20/89.

377-015 066065 "Test for androgenic and anabolic activity in the rat". Special study by Institute of Biological Sciences. Castrated male rats were dosed subcutaneously daily for 7 days with 0.2 to 2 mg/rat total dose of diflubenzuron. Positive controls received a total dose of 0.5 or 2.0 mg testosterone. Endpoints were tissue weight/body weight ratios of seminal vesicles and ventral prostate (for androgenic activity), and tissue weight/ body weight ratio of the levator ani (to determine anabolic activity). There was no tissue weight ratio change in measured parameters for diflubenzuron at either dose, however 0.5 mg testosterone markedly increased ratios for all three tissues. This study was negative, and demonstrated that diflubenzuron was less than 1/4 as potent as testosterone in this assay. No worksheet. One-liner by Aldous, 9/20/89.